

# Late laying hens deposit dietary antioxidants preferentially in the egg and not in the body

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**Primary Audience:** Researchers, Nutritionists, Feed Manufacturers, Poultry Processing Managers, Egg Producers

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## SUMMARY

The allocation of nutrients in the metabolism of laying hens favors the egg over the body. It is unclear whether this programming also includes micronutrients and antioxidants. This was tested with five by seven 76-wk-old Heisdorf & Nelson Brown Nick layers. They were fed a basal diet low in antioxidants either unchanged (control) or supplemented with 40 IU of  $\alpha$ -tocopherylacetate/kg of diet (vitamin E) or 2.5% chokeberry pomace, rose hip, or sage in the last 4 wk before slaughter. The traits measured were subjected to ANOVA. The additives did not affect ADFI, performance, egg quality, carcass, or meat quality. Yolk tocopherol contents were higher with vitamin E and sage compared with control and rose hip treatments. Oxidative stability was more affected in egg yolk powder than in the meat. After 12 wk of storage, yolks from vitamin E-treated hens were lowest in TBA levels (5.0 mg of malondialdehyde/kg), followed by chokeberry (8.7) and sage (8.8). Rose hip (18.1) and control (18.9) treatments were similar. In meat, TBA was slightly decreased after 9 d of storage in meat from chokeberry- and sage-supplemented hens (contrast analysis). In conclusion, antioxidant deposition to the egg seems to be part of the genetic determination of the hen. Adding antioxidants is therefore interesting for layer nutrition in general, and herbal additives allow performing this in a natural way. The programming for partitioning, however, renders the strategic feeding of antioxidants before slaughter, with the goal to increase oxidative stability of spent hen meat rather inefficient.

**Key words:** spent hen, meat, egg, rose hip, chokeberry, sage, antioxidant

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## DESCRIPTION OF PROBLEM

Laying hens are genetically determined to maintain as best as possible the most favorable macro- and micronutrient composition of the egg. This is an important homeorhetic principle guaranteeing the survival of the offspring. In case of severe deficiency of energy and nutrients, laying performance will decline, whereas the

composition of the egg content will not change. However, long-term breeding efforts have genetically programmed laying hens to partition most energy and nutrients to the egg and as few as possible into body reserves [1, 2], thus possibly even affecting the hen's overall fitness [3]. This also happens in case of a certain deficiency of, for instance, energy [4] and protein [3]. In addition, housing measures such as lighting pro-

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grams further intensify egg production. Antioxidants are important in both metabolism [5] and in reproduction of female poultry [6], and their protective action is also needed by the embryo [7]. This increased the attention to their role in layer diets [8], but more related to whether extra antioxidants would increase laying performance [3]. However, if maintaining a certain level of antioxidants in the egg is also part of the overall homeorhetic principle outlined previously, they should be preferentially deposited in the egg and not in the meat in case of limited supply and high laying performance. Otherwise, feeding diets rich in antioxidants at the end of the egg production cycle would help in marketing of the carcasses of spent hens. Valorization of spent hen meat is increasingly performed by its use as a raw material for producing specific sausages and other meat products where antioxidant stability is an issue [9, 10]. This is in response to changes in cooking habits in industrialized countries, which makes it increasingly difficult to sell these birds for the traditional consumption purpose as stewing hens [11].

Antioxidants used in the technical processing of the meat are known for their effectiveness in increasing the oxidative stability in spent hens. This also includes specific herbs. For instance, Lee et al. [12] demonstrated a significant reduction in lipid oxidation in breakfast sausages produced from spent hen meat when different antioxidant extracts from plants were added during processing. However, using plant material is always associated with a hygienic risk for meat processors [13]. This approach is not possible for that part of the spent hen carcasses which is still sold for stewing anyway, and the comparative advantage of simultaneously prolonging the oxidative shelf life of the egg by dietary intervention is lost as well. Dietary synthetic vitamin E was shown to be effective in prolonging the oxidative shelf life of eggs [6, 14]. Similarly, plants with antioxidant activity, such as rosemary and sage, were successfully used as feed additives [14, 15] and may be of interest as natural means to increase the oxidative stability of both spent hen meat and egg products. Thus, it would be helpful when the bioactive plant materials were also effective in the hens via a dietary intervention. This approach seems promising, as it has been demonstrated to augment the re-

spective oxidative stability in studies focusing on either meat or eggs before. For instance, in broilers, the antioxidant properties of the meat were found to be increased when supplementing diets with rosemary (*Rosmarinus officinalis*) and, to a lesser degree, with chokeberry pomace (*Aronia melanocarpa*) and rose hip (*Rosa canina*) [16]. Similarly, a significant reduction in breast muscle oxidation was reported when a combination of papaya leaves and vitamin D<sub>3</sub> was fed to spent hens a few weeks before slaughter [17]. Sage (*Salvia officinalis*) also has antioxidant activity [18] and has been shown to exhibit its favorable effects when fed to broilers [15]. In laying hens, a positive effect of dietary rosemary on egg yolk stability has been demonstrated before [14]. Also, oregano (*Origanum vulgare*), curcuma (*Curcuma longa*), and thyme (*Thymus vulgaris*) had a positive effect against lipid oxidation of egg yolk [6].

The objective of the present study was to test dietary antioxidants and plants rich in antioxidants for their efficiency in simultaneously increasing the oxidative shelf life in meat and eggs of late-laying hens based on the following hypotheses: (1) antioxidants in feed increase the oxidative stability of the meat and the eggs of hens in their late-laying phase, and (2) this increase happens to a similar degree in meat and eggs. (3) Effective natural additives may increase the oxidative stability of meat and eggs to the same extent as synthetic vitamin E. It was also tested whether or not the dietary measures had effects on performance, meat yield, and general egg and meat quality.

## MATERIALS AND METHODS

### *Birds, Diets, and Experimental Design*

For the experiment, 35 Heisdorf & Nelson Brown Nick laying hens were used. They were 76 wk old and caged individually (80 × 80 × 80 cm) with ad libitum access to feed and water (for more details see Loetscher et al. [19]). The room temperature was kept at 20°C and the light program provided 14L:10D. The experiment was approved by the cantonal veterinary office of Zurich, Switzerland (approval no. 191/11). All hens were fed for a 2-wk adaption period on a basal diet that was not supplemented with vitamin E. Subsequent to the adaptation period, a

4-wk experimental period followed where 5 diets differing in supplementation were fed. In a complete randomized design, the 35 hens were allocated to these 5 groups with 7 birds each such that each group had a similar average laying performance and BW. The 5 experimental diets consisted either of the basal diet (control) or of the basal diet supplemented with 40 IU of DL- $\alpha$ -tocopherylacetate/kg (Rovimix E 50 SD [20]; vitamin E) or 2.5% of natural additives, namely chokeberry, rose hip, or sage. The level of the natural additives was equal to that chosen in a previous experiment, which had been sufficient to result in enhancement of antioxidant properties of broiler meat [16]. The natural additives were represented by leaves (sage), fruits (rose hip), and pomace (chokeberry; i.e., the residue of the berries from juice production). All additives were dried at 50°C and ground through a 0.75-mm screen (mill model SM1 [21]), before being mixed into the diets. The completed diets were then separately steam pelleted through a 2.5-mm matrix mounted on a laboratory pelleting press [22]. The ingredient composition of the basal feed mixture, which was the basis of all experimental diets, is presented in Table 1.

### Data and Sample Collection

Weekly, ADFI and BW were recorded. Eggs were collected and weighed daily for each hen individually. Representative excreta samples were collected on 3 consecutive days at the beginning of wk 4. For this, the sand bath was removed from the cages. The combined samples per hen were stored at -20°C before being freeze-dried [23]. Diets were sampled on the d 1 of the 3-d excreta collection period. Diets and excreta were ground through a 0.75-mm screen on a centrifugal mill (model ZM1 [21]). The last 5 eggs laid in the experiment (wk 4) were sampled and stored at 4°C until later analysis.

At the end of the experiment, all hens were slaughtered by stunning and subsequent bleeding of the cervical artery. Internal organs and abdominal fat were excised and weighed. Carcass weight was defined as the eviscerated body without feathers, feet, head, neck, and abdominal fat. Dressing percentage described the proportion of carcass weight in BW. Breast muscles were prepared and weighed without skin. The

entire thighs with skin and bones were excised after dislocating the leg from the hip joint. Breast muscles, thighs, abdominal fat, and liver were weighed, vacuum packaged and stored at -20°C until further analysis.

### Laboratory Analysis

Standard procedures [24] were used for the analysis of feeds (diets and additives) and excreta. Dry matter was determined with a thermo gravimetric device (model TGA-500 [25]). A C/N analyzer (type CN-2000 [25]) was used to determine N, and CP was calculated as  $6.25 \times N$ . Exclusively in the feed, EE was determined with a Soxhlet extraction system (model B-811 [26]). In feeds only, NDF and ADF, both corrected for ash content, were determined according to Mertens et al. [27] and AOAC [24] method #973.18, respectively, on a Fibertec System M [28]. An amount of 100  $\mu$ L of  $\alpha$ -amylase [29]

**Table 1.** Ingredient composition of the basal diet<sup>1</sup>

Ingredient	%, as fed
Corn	38.9
Wheat	17.0
Soybean meal	14.7
Sorghum (milocorn)	5
Wheat bran	3.5
Soybean oil <sup>2</sup>	3.2
Corn gluten	2.5
Oat husks	2.0
Limestone grit	5.0
Calcium carbonate	4.2
Dicalcium phosphate	1.1
Vitamin-mineral premix <sup>3</sup>	0.5
Sodium bicarbonate	0.3
Sodium chloride	0.15
DL-Met	0.1
L-Lys	0.1
Celite <sup>4</sup>	1.75

<sup>1</sup>The nutrient and energy contents, as calculated, were AME, 2,800 kcal/kg; CP, 15.1%; Lys, 0.72%; Met and Cys, 0.63%; calcium, 4.0%; total and available phosphorus, 0.53 and 0.29%, respectively.

<sup>2</sup>Without technical antioxidants.

<sup>3</sup>The vitamin-mineral premix provided per kilogram of feed: Ca, 1.25 g; Mn, 80 mg; Zn, 60 mg; Mg, 50 mg; Fe, 50 mg; Cu, 6 mg; I, 310  $\mu$ g; Se, 200  $\mu$ g; retinol, 12,500 IU; cholecalciferol, 3,000 IU; choline, 100 mg; betaine, 99.9 mg; niacin, 40.5 mg; pantothenic acid, 15 mg; riboflavin, 5 mg; pyridoxine, 3.95 mg; menadione, 2.6 mg; thiamine, 2 mg; folic acid, 1.49 mg; biotin, 200  $\mu$ g; cyanocobalamin, 20  $\mu$ g.

<sup>4</sup>[82].

was added for NDF determination. Gross energy was measured with a bomb calorimeter system C700 with cooler C7002 [30]. Insoluble ash was used as indigestible marker. For that purpose, celite was added to the basal diet (Table 1) to increase the content of hydrochloric-acid insoluble ash (**IA**) in feed and excreta and thus increase the accuracy of the method. In diets and excreta, IA was analyzed by boiling the samples in 100 mL of 4 M HCl for 30 min and quantitative filtration of the remaining slurry through ash-free filter paper [31, 32]. The slurry was washed free of acid and dry-ashed for 6 h at 550°C. The results were used to calculate the coefficients of metabolizabilities of nitrogen and energy and the actually realized dietary AME contents. The corresponding equations [33] used were:

1. metabolizability (nitrogen / energy) =  $1 - [( \text{concentration of IA in feed} / \text{concentration of IA in excreta} ) \times ( \text{nitrogen or energy concentration in excreta} / \text{nitrogen or energy concentration in feed} )]$ ; and
2. measured AME (kcal/kg) = gross energy content (kcal/kg)  $\times$  energy metabolizability.

In the diets and the natural feed additives, total phenol content was measured by the Folin-Ciocalteu method [34], but gallic acid was used as a standard. Prior to that, 100 mg of sample were extracted for 20 min in 5 mL of 70% acetone [29] in an ultrasonic water bath [35]. This procedure was repeated twice and the supernatants were combined and mixed thoroughly before further analysis.

The 4 forms of tocopherol ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) were analyzed in the diets and the natural feed additives. First, the samples were saponified with KOH [36] for 30 min at 95°C. The actual analysis was conducted on a normal-phase HPLC model 2695 equipped with a fluorescent detector [37] on a Nukleosil 100–5 CC125/4 column [38, 39].

Two in vitro methods were used to determine the direct antioxidant potential of the herbal additives. For the radical scavenging activity (**RSA**), the same extraction procedure as that used for the phenol analysis was applied, except that 50% methanol was used as a solvent instead. The free radical, 2,2-diphenyl-

1-picrylhydrazyl (**DPPH**<sup>•</sup> [29]) was used. The analysis was performed according to Mansouri et al. [40]. From the combined supernatant, 50  $\mu$ L were added to 950  $\mu$ L of 0.12 mM methanolic DPPH<sup>•</sup> solution. After 30 min in the dark, the absorbance was measured at 515 nm using a UV-Vis double-beam scanning spectrophotometer model UV-160A [41]. The amount of sample (mg of sample/mg of DPPH<sup>•</sup>) necessary to reduce half of the initial DPPH<sup>•</sup> concentration was defined as EC<sub>50</sub>. In the second method, the extent to which the induction time of oleic acid (i.e., the time until secondary oxidation products were analytically detected) could be prolonged with the addition of the natural feed additives was measured with a Rancimat model 697 [42]. For that, 75 mg of ground material was added to 2.5 g of oleic acid [29] and incubated at 100°C with an air flow of 20 L/h. From the results, the Rancimat antioxidant factor (**RAF**) was calculated as the ratio to the value found with oleic acid alone.

The last egg collected was subjected to the analyses described herein on the day of collection. An electronic hardness tester type PTB 301 [43] was used to assess shell strength. The absolute weights of the whole egg, yolk, and shell were recorded. For that purpose, the egg shells were cleaned and the membranes were removed. Then they were dried for 24 h at 80°C before weighing. The weight of the egg white was calculated by difference. The average shell thickness was estimated as the mean of 3 measurements (both poles and equator) performed with a thickness-testing instrument [44]. Haugh units were calculated as  $100 \times \log (\text{measured height of the egg white} - 1.7 \times \text{egg weight}^{0.37} + 7.6)$  [45]. The yolk color was assessed by subjective scoring on the 15-grade scale of a yolk color fan [20] and objectively by the L\*, a\*, b\* color space (diffuse illumination/0° viewing angle; measuring area, 8 mm) using a chromameter model CR-300 [46]. The yolks from the last 5 eggs were combined and lyophilized. This material was homogenized with a mix chopper [47]. The resulting yolk powder was used to determine tocopherols as described above for the feeds, and oxidative stability using TBA reactive substances (**TBARS**) [48]. The TBARS were analyzed after 0, 4, 8, and 12 wk of storage in the open at 20°C and at 12 h/d of light

exposure (1,000 lx). The results were given as malondialdehyde equivalents in milligrams per kilogram of yolk powder.

Directly upon dissection of the carcasses, color on top of the prepared breast muscle and of the right liver lobe was measured using the same settings on the chromameter as for yolk color. For the other analyses, the previously frozen tissue samples were thawed over night at 4°C before analysis. The right breast muscle was used to conduct various meat quality measurements. The pH was measured with a pH340/Set-1 [49]. To determine thawing and cooking loss, the breast muscle was weighed before and after being thawed and again after being cooked, to a core temperature of 74°C in a water bath. Shear force and maximum shear energy were subsequently measured with a Volodkevich device [50] mounted on a texture analyzer model TA-HD [51]. This device was used instead of the more commonly applied Warner-Bratzler shear blade because its 2 wedges simulate the action of the molars [50]. This is important to assess the tenderness of inherently tough meat, such as that of spent hens. The left breast muscle was homogenized to measure the contents and profiles of tocopherol analogous to feed and egg yolk samples. In this material water-binding capacity (WBC) was also determined [52, 53]. In detail, 10 g of homogenized meat sample were mixed on a vortex for 20 s with 15 mL of 0.6 M aqueous sodium chloride [29] in a 50-mL centrifugation tube. The mixture was stored at 4°C for 15 min before mixing for another 20 s. Thereafter, the homogenate was centrifuged at 4°C at  $3,000 \times g$  for 25 min. The supernatant was weighed and the WBC was expressed as percentage of the originally added liquid retained. The content of connective tissue was calculated as  $8 \times$  hydroxyproline (method 318.1 Swiss Food Manual [54]). Hydroxyproline was determined according to the method 317.1 [54]. Briefly, the meat sample was dissolved in sulfuric acid [36] and hydroxyproline was oxidized with chloramine T trihydrate [29]. The oxidation product was mixed with 4-dimethylaminobenzaldehyde [29] to form a color complex that was measured photospectrometrically at 560 nm.

In the thigh muscle, TBARS and i.m. fat content were measured. For this, skin and adherent fat were removed. The meat was deboned and

then homogenized. In the homogenate, EE was determined after hydrolyzation of the sample in 4 M HCl (method 319 [54]). The TBARS in meat covered by a transparent film were measured as in the egg yolk powder, but on d 0, 3, 6, and 9 of storage at 4°C and 12 h/d of light exposure (1,000 lx).

The induction time of abdominal fat was determined on the same Rancimat as the RAF. First, the abdominal fat was cut into pieces (1 cm<sup>3</sup>) and melted for 30 min in a drying cabinet at 60°C. Thereof, 2.5 g were subjected to incubation at 105°C and an air flow of 20 L/h.

### Statistical Analysis

Outliers were defined as values exceeding the mean value of the variable by more than 3 times the SD and were excluded before statistical analysis. The experimental data were subjected to ANOVA using the MIXED procedure of SAS 9.3 [55] with the individual bird as experimental unit. For the TBARS data, the effect of day or week of storage for meat and egg yolk samples, respectively, was included by a repeated measurement statement with covariance structure as the variance component. Orthogonal contrasts were used to compare the treatment means of the TBARS data across all time points to control treatment, and significance was presented as  $P_C$  in text. Multiple comparisons among treatment means were made with Tukey's procedure. For all data,  $P < 0.05$  was considered as statistically significant.

## RESULTS AND DISCUSSION

### *Effect of Vitamin E and the Herbal Additives on Diet Composition and Performance*

Compared with the basal diet, the herbal additives were richer in fiber and poorer in CP, with the differences being particularly pronounced with rose hip, followed by chokeberry (Table 2). Chokeberry contained the most total phenols, followed by sage and rose hip. The phenol content was lowest in the basal diet, where the natural content of tocopherols was also low. Rose hip was richest in total tocopherols, mainly because of an obvious richness in the  $\gamma$ -form compared with the other natural additives. Sage had by far the highest antioxi-



**Table 2.** Analyzed concentrations of nutrients and secondary compounds in the complete diets and the natural additives (per kilogram, as fed) as well as the antioxidant activity of the natural additives as determined by 2 in vitro test systems<sup>1</sup>

Item	Complete diet					Natural additive		
	Basal diet	Vitamin E	Sage	Chokeberry	Rose hip	Sage	Chokeberry	Rose hip
DM (g)	901	913	914	916	907	913	931	930
Organic matter (g)	773	773	778	781	770	828	912	888
Gross energy (kcal)	3,646	3,785	3,701	3,756	3,667	4,838	4,556	4,325
CP (g)	163	166	165	165	161	99.6	78.3	65.6
EE (g)	47.2	46.5	48.9	51.2	51.0	70.4	34.8	38.0
NDF (g)	111	106	115	113	115	287	340	482
ADF (g)	41.0	42.7	50.6	47.9	51.7	256	226	382
Total phenols (g)	6	6	8	7	7	104	117	72
Tocopherols (mg)								
Total	49	77	61	53	48	113	118	134
$\alpha$	46	73	44	44	41	108	101	98
$\beta$	n.d. <sup>2</sup>	n.d.	0.24	0.07	n.d.	0.71	9.81	0.19
$\gamma$	0.68	0.69	10.77	3.61	2.23	1.93	3.20	34.10
$\delta$	3.07	3.26	6.31	5.33	4.42	2.26	4.36	2.39
Rancimat antioxidant factor <sup>3</sup>	— <sup>4</sup>	—	—	—	—	42.51	3.87	1.93
Radical scavenging activity, EC <sub>50</sub> <sup>5</sup>	—	—	—	—	—	1.31	2.05	2.59

<sup>1</sup>Values are the mean of 2 determinations per sample.<sup>2</sup>n.d. = not detected.<sup>3</sup>X-fold prolongation of induction time of 2.5 g of oleic acid with 75 mg of herbal additives.<sup>4</sup>Not analyzed.<sup>5</sup>EC<sub>50</sub> = amount of sample necessary to decrease the initial DPPH concentration by half.

dant activity as measured in the 2 in vitro test systems (RAF, RSA). Major antioxidant phenolic compounds of sage include rosmarinic acid, carnosic acid, and carnosol [56]; in chokeberry pomace these are chlorogenic acid, neochlorogenic acid, polymeric procyanidins, cyanidin-3-galactoside, and cyaniding-3-arabinoside [57]; and in rosehip these are hydroxycinnamic acids, catechin, quercetin, and kaempferol [58].

The addition of the herbal additives at 2.5% caused no substantial changes in the nutrient composition of the complete diets except for the ADF. It was higher in all plant-supplemented diets (4.8–5.2%) compared with in the basal diet (4.1%; Table 2). The supplemented diets were richer in phenols (diets supplemented with natural additives) and tocopherols (except rose hip). It should be stated that, due to the comparably low concentrations in the complete diets, the accuracy of these values is limited (discussed in [16]). The RAF and RSA could not be analyzed in the complete diets because the antioxidant concentration was too low to be assessed with these test systems.

No differences ( $P > 0.05$ ) in ADFI, BW, FCR, and laying performance were observed across all supplementation treatments (Table 3). However, a supplementation effect ( $P < 0.05$ ) on energy metabolizability and realized dietary AME content was noted. In both variables, the rose hip treatment resulted in unfavorably lower levels. Vitamin E as well as chokeberry treatments caused favorable responses in these 2 variables. Although rose hip had adverse effects on energy metabolizability, the nonsupplemented control was also inferior to the vitamin E-supplemented group regarding measured AME. Because the vitamin E and control diets only differed in their tocopherol content and vitamin E is a potential antioxidant, this appears to be linked to a decreased lipid oxidation during feed storage. Actually, Racanicci et al. [59] reported a lower AME when oxidized fat was fed to growing broilers, which also led to a depressed growth rate [60], an effect which could be reversed by adding ethoxyquin, a synthetic antioxidant. However, in the sage group, an equally low AME content was found as in the control

**Table 3.** Influence of vitamin E (40 mg/kg) and natural additives (2.5%) as feed supplements on performance and metabolizability of late laying hens in comparison with an unsupplemented control treatment<sup>1</sup>

Treatment diet	Control	Vitamin E	Sage	Chokeberry	Rose hip	SEM	P-value
n	7	7	7	7	7		
ADFI (g/bird)	110	103	99	103	107	5.8	0.67
Final BW <sup>2</sup> (kg)	1.90	1.90	1.89	1.88	1.93	0.089	1.00
Laying performance (eggs/d)	0.811	0.781	0.806	0.872	0.837	0.123	0.99
FCR (g of feed/g of egg)	1.79	1.69	1.74	1.83	1.77	0.068	0.65
Metabolizability <sup>2</sup>							
Nitrogen	0.374	0.342	0.383	0.425	0.362	0.0444	0.76
Energy	0.738 <sup>ab</sup>	0.746 <sup>a</sup>	0.723 <sup>ab</sup>	0.742 <sup>a</sup>	0.707 <sup>b</sup>	0.0077	0.007
Measured AME (kcal/kg) <sup>2</sup>	2,691 <sup>bc</sup>	2,823 <sup>a</sup>	2,674 <sup>bc</sup>	2,786 <sup>ab</sup>	2,593 <sup>c</sup>	27	<0.001

<sup>a-c</sup>Values in the same row with different superscript are significantly different ( $P < 0.05$ ).

<sup>1</sup>Control, birds fed a diet without extra vitamin E or natural additives.

<sup>2</sup>Determined in wk 4 of the experiment.

group despite the sage's strong antioxidant properties. Still, it is possible sage had a low enough dietary value that even a supplementation level of 2.5% was sufficient to counterbalance a positive effect on feed lipid oxidation.

On average during the experimental period, the hens daily consumed 104 g of feed. The mean FCR (g of feed/g of egg) ranged between 1.74 and 1.83. Laying performance across the experimental period covered a range from 0.78 to 0.87 eggs/d.

Overall, vitamin E and the herbal additives obviously did not substantially change gross nutrient composition of the experimental diets and performance of the hens. Due to their richness in tocopherols and phenols, the herbal additives contributed to oral antioxidant supply of the hens, though this resulted in much lower tocopherol supplementation levels than the vitamin E treatment (per kilogram of diet: 40 mg of  $\alpha$ -tocopherol vs. 3.0, 3.4, and 2.8 mg/kg of chokeberry pomace, rose hip, and sage). Evidence from a previous broiler experiment indicates a possible enhancement of performance with rose hip feeding [16]. Therefore, it seems that laying hens do not respond to dietary rose hip as the physiologically and metabolically different broilers do.

#### ***Effect of Vitamin E and the Herbal Additives on Egg, Carcass, and Meat Quality***

The supplementation treatments did not influence egg gross composition, shell stability, or yolk color (Table 4). Weight, shell proportion, and shell strength of the eggs were not affected

( $P > 0.05$ ) by treatment, either. This illustrates that the feeding strategies did not result in any unfavorable side effects in egg quality.

Among the carcass traits, only the relative spleen weight was affected by the supplementation treatments (Table 5); it was lower ( $P < 0.05$ ) in the vitamin E-treated hens than the control and sage groups. It has been shown in growing rats that spleen-to-BW ratio is an indicator of vitamin E status, and the ratio is higher in case of vitamin E deficiency [61]. However, considering the current recommendations to prevent vitamin E deficiency in laying hens which are in a range of 12 to 41 [62] and 30 mg/kg [63], requirements were covered also by the unsupplemented control diet. Thus, it was unlikely that the hens would develop any severe deficiency in the present study. Additionally, breast muscle tocopherol status did not differ between treatments (Table 6). Thus, the difference in spleen-to-BW ratio found with 2 treatment groups was probably not caused by a severe vitamin E deficiency in the respective diets.

The traits describing physicochemical meat quality also remained unaffected ( $P > 0.05$ ) by treatment (Table 6). This included the traits describing water-holding capacity (thawing and cooking losses as well as WBC), tenderness (Volodkevich shear data and content of connective tissue), i.m. fat content, and color of the meat. Although the main focus of the present study was on the antioxidant principles, a possible effect on the physicochemical properties of the meat could not be excluded. Evidence exists that bioactive compounds, such as the meat-tenderizing papain, may be transferred from the

**Table 4.** Influence of vitamin E (40 mg/kg) and natural additives (2.5%) as feed supplements on the quality of the last egg per hen laid in the experiment by late laying hens in comparison with an unsupplemented control treatment

Treatment diet	Control	Vitamin E	Sage	Chokeberry	Rose hip	SEM	P-value
n	6	6	7	6	6		
Egg weight (g)	67.3	70.2	64.2	66.0	64.5	2.42	0.42
Egg constituents (%)							
Yolk	24.3	23.4	25.0	25.5	23.7	0.87	0.40
White	67.3	66.7	65.5	65.0	66.9	1.00	0.41
Shell	8.42	9.85	9.47	9.53	9.43	0.387	0.14
Shell strength (N)	25.7	34.7	37.3	33.6	35.1	4.03	0.34
Shell thickness (µm)	35.9	41.5	39.6	39.9	39.5	1.57	0.19
Haugh units	89.0	76.0	79.5	80.4	84.5	4.43	0.32
Yolk color							
Yolk color fan <sup>1</sup>	10.5	10.8	10.8	11.0	11.1	0.31	0.72
Lightness (L*)	50.6	52.0	51.2	51.1	50.0	0.93	0.63
Redness (a*)	4.73	4.17	4.77	5.54	5.66	0.535	0.28
Yellowness (b*)	30.5	32.8	32.2	31.5	30.6	1.00	0.46
Tocopherol content (mg/kg of egg yolk powder)							
Total	38.9 <sup>c</sup>	162.8 <sup>a</sup>	91.3 <sup>b</sup>	68.7 <sup>bc</sup>	54.6 <sup>c</sup>	7.37	<0.001
α	11.1 <sup>c</sup>	135.9 <sup>a</sup>	60.3 <sup>b</sup>	29.8 <sup>c</sup>	21.5 <sup>c</sup>	5.80	<0.001
β	1.65 <sup>b</sup>	1.68 <sup>b</sup>	1.91 <sup>b</sup>	3.14 <sup>a</sup>	2.09 <sup>b</sup>	0.238	<0.001
γ	21.5 <sup>b</sup>	21.4 <sup>b</sup>	26.3 <sup>ab</sup>	31.5 <sup>a</sup>	26.6 <sup>ab</sup>	2.13	0.010
δ	4.68 <sup>a</sup>	3.89 <sup>ab</sup>	2.74 <sup>b</sup>	4.31 <sup>ab</sup>	4.31 <sup>ab</sup>	0.391	0.018

<sup>a-c</sup>Values in the same row with different superscript are significantly different ( $P < 0.05$ ).

<sup>1</sup>[20].

diet to the meat by yet unknown pathways, as could be demonstrated by feeding diets containing 10% papaya leaves [64]. However, the herbal additives applied in the present study did not provide comparable tenderness relevant bio-active principles.

#### ***Effect of Vitamin E and the Herbal Additives on Oxidative Stability of Eggs and Meat***

A 4-fold increase ( $P < 0.05$ ) in tocopherol content of the egg yolk was observed in hens in the vitamin E group compared with egg yolks from the control group (Table 4). Also, sage addition resulted in a clearly ( $P < 0.05$ ) higher total tocopherol content, albeit only to a 2.3-fold level of control. This effect could be due to a vitamin E-sparing effect of the antioxidant components in sage in the intestine. Thus, more vitamin E is available and absorbed in the gut compared with a diet free of antioxidants other than vitamin E. This effect was demonstrated before in chicken [65] and rats [66]. The tocopherol contents of egg yolks obtained with the rose hip and chokeberry diets resulted in intermediate values between the control and the vitamin E groups.

The largest changes in the yolk happened with α-tocopherol, but the chokeberry diet was also efficient in increasing β- and γ-tocopherol in egg yolk. This was consistent with the comparatively high β-tocopherol content of the chokeberry pomace, whereas this was not the case with γ-tocopherol. The sage treatment resulted in the lowest δ-tocopherol content of the yolk, the content of which was low in sage as well. Unlike in the egg yolk, even the vitamin E and the sage treatment did not result in an increase ( $P > 0.05$ ) of total, α-, and δ-tocopherol contents of the breast muscle and no β- and γ-tocopherols were detected in any of the samples (Table 6). It has, however, been shown earlier in broilers that dietary α-tocopherol at a high dosage, either given pure or via herbal additives, can also elevate breast muscle tocopherol content [16]. This indicates that a fundamental difference between growing and adult animals exists regarding vitamin E accumulation in the body tissue.

All supplements except rose hip resulted in a clear increase in the protection of the lipids present in the egg yolk against oxidation ( $P < 0.05$ ), as is obvious from the development of TBARS with time (Figure 1). The analysis of the con-



**Table 5.** Effect of vitamin E (40 mg/kg) and natural additives (2.5%) as feed supplements on carcass and organ weights of late-laying hens in comparison with an unsupplemented control treatment

Treatment diet	Control	Vitamin E	Sage	Chokeberry	Rose hip	SEM	P-value
n	7	7	7	7	7		
Carcass weight (kg)	1.18	1.17	1.18	1.20	1.22	0.078	0.99
Dressing percentage	61.7	61.4	61.8	63.4	63.0	1.23	0.71
Proportion of carcass weight (%)							
Breast muscle	16.5	16.4	16.4	16.2	16.8	0.45	0.92
Thigh <sup>1</sup>	31.7	31.2	31.5	31.2	30.7	0.64	0.84
Abdominal fat	5.48	5.09	4.32	5.26	4.73	0.866	0.89
Pancreas	0.309	0.360	0.350	0.286	0.333	0.0220	0.14
Liver	2.72	2.66	2.95	2.64	2.72	0.141	0.56
Heart	0.568	0.653	0.612	0.607	0.628	0.0285	0.33
Spleen	0.202 <sup>a</sup>	0.145 <sup>b</sup>	0.198 <sup>a</sup>	0.164 <sup>ab</sup>	0.161 <sup>ab</sup>	0.0118	0.006
Stomach	2.84	3.10	2.94	2.86	2.91	0.219	0.92

<sup>a,b</sup>Values in the same row with different superscript are significantly different ( $P < 0.05$ ).

<sup>1</sup>Weighted with skin and bones.

trasts against the control treatment confirmed these findings. Different from the egg yolk, no significant differences occurred in the multiple comparisons of the TBARS treatment means

in thigh meat. However, the contrast analysis of the treatments against control revealed a clearly slower oxidation when chokeberry and sage were added to the layer diet ( $P_C = 0.001$

**Table 6.** Effect of vitamin E (40 mg/kg) and natural additives (2.5%) as feed supplements on meat quality (measured in breast muscle unless indicated otherwise) of late-laying hens in comparison with an unsupplemented control treatment

Treatment diet	Control	Vitamin E	Sage	Chokeberry	Rose hip	SEM	P-value
n	7	7	7	7	7		
pH	5.72	5.72	5.68	5.76	5.74	0.029	0.47
Substance loss (%)							
Thawing	4.02	4.27	3.32	3.78	3.55	0.551	0.77
Cooking	12.9	12.1	13.0	13.1	13.1	1.20	0.97
WBC <sup>1</sup> (%)	49.8	52.1	44.9	53.7	54.5	5.36	0.72
Volodkevich shear values							
Maximal force <sup>2</sup> (N)	24.1	25.8	25.7	25.6	29.2	2.86	0.79
Total energy (mJ)	85.3	86.7	87.3	89.6	96.5	6.11	0.72
Connective tissue (g/kg)	0.900	0.871	0.927	0.899	0.934	0.0629	0.96
I.m. fat (% of thigh muscle)	4.75	5.18	5.15	4.69	4.82	0.377	0.83
Color							
Breast							
Lightness (L*)	58.7	58.0	57.4	58.4	55.9	1.90	0.85
Redness (a*)	0.508	0.424	0.549	0.106	0.566	0.2428	0.66
Yellowness (b*)	1.53	0.56	1.52	1.09	0.88	0.438	0.47
Liver							
L*	41.5	39.8	41.3	41.8	39.7	0.91	0.32
a*	14.8	14.6	14.5	14.3	15.2	0.42	0.70
b*	14.7	13.1	16.1	14.5	12.9	1.60	0.64
Tocopherol content (mg/kg)							
Total	1.66	1.64	1.47	1.93	1.58	0.231	0.72
α	0.79	0.99	0.80	0.96	0.80	0.132	0.70
δ	0.618	0.641	0.811	0.945	0.778	0.1137	0.26
Rancimat induction time (h; abdominal fat)	2.70	3.11	3.39	3.09	2.92	0.424	0.83

<sup>1</sup>WBC = water-binding capacity (i.e., percentage of water that the minced meat could retain in addition after centrifugation).

<sup>2</sup>First maximum.

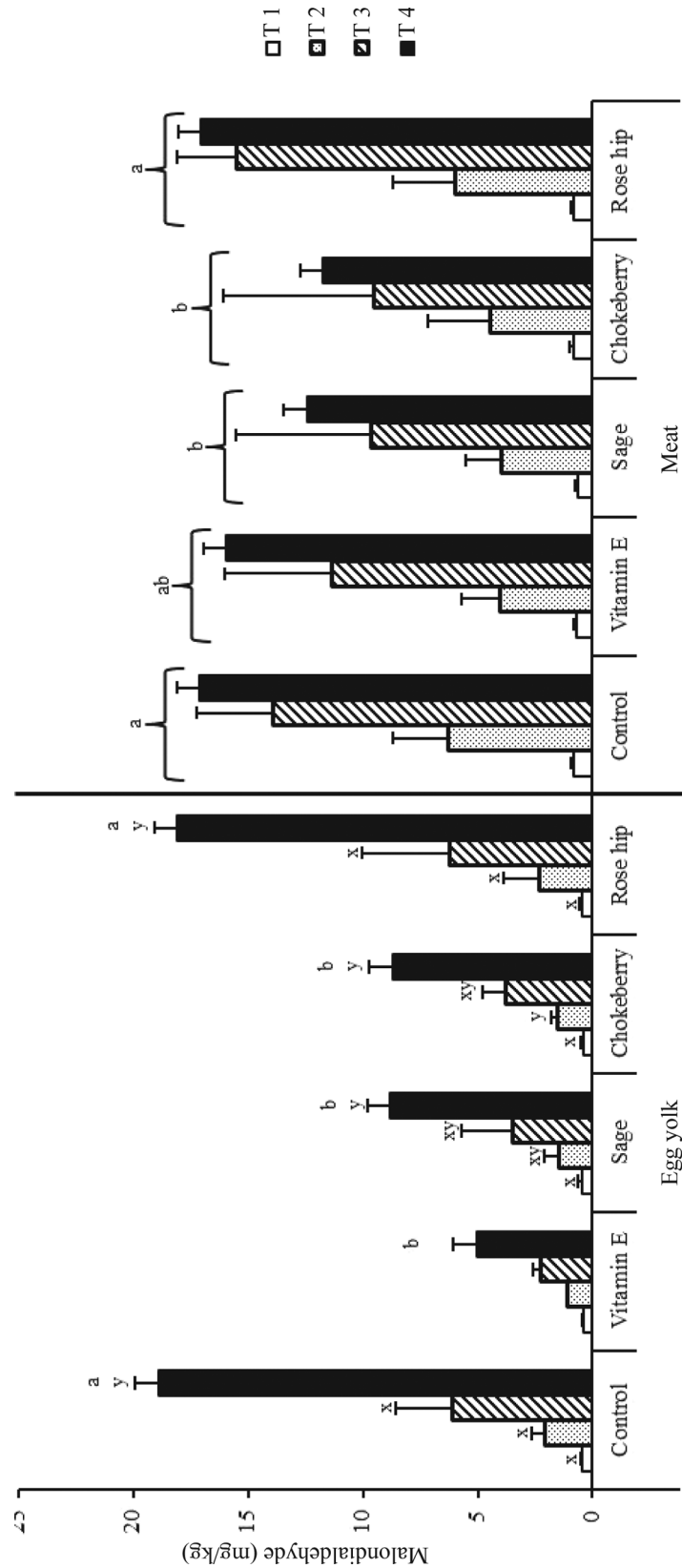
and 0.002, respectively), whereas the addition of rose hip had no effect ( $P_C = 0.73$ ) and that of vitamin E resulted only in a tendency ( $P_C = 0.085$ ). When the TBARS results were related to units of i.m. fat content of the thigh meat (data not shown in figure), vitamin E addition significantly ( $P_C = 0.024$ ) delayed oxidation compared with the control. The induction time of the abdominal fat (i.e., the time until rancidity occurred) was not affected by treatment (Table 6).

The antioxidant property of vitamin E is well documented [14, 67, 68]. However, all 3 natural products employed as dietary additives are also known for their direct antioxidant properties. Sage, in particular, has proven its effectiveness in vitro [18, 69]. Chokeberry and rose hip are also considered potential antioxidants [70, 71]. Accordingly, also the batches of the herbal additives tested in the present study exhibited in vitro antioxidant properties (Table 2). Sage was clearly superior to chokeberry and especially rose hip in RAF and RSA. Concerning the tocopherols [72, 73], the 3 natural additives provided about 3-fold higher levels compared with the basal diet. All 3 additives have been tested in broilers before—sage by Lopez-Bote et al. [15], chokeberry pomace and rose hip by Loetscher et al. [16]—where they have promoted oxidative protection in the meat. However, we do not know of any research testing the effect of these herbal additives in spent hen meat.

Part of the antioxidant effect found with the herbal additives may have resulted from the tocopherols they provided. Yet, not all tocopherol forms are equally effective antioxidants [74]. The most effective form,  $\alpha$ , was the most prevalent in all herbal additives; but, in eggs and meat, other forms of tocopherol were substantial in content as well. In the egg yolk, the concentration of  $\alpha$ -tocopherol was 2 and 4.5 times lower with the sage and chokeberry treatments, respectively, in comparison with the vitamin E treatment. This was opposite for the  $\gamma$ -tocopherol content, where the concentration in the yolk was as high or higher with sage and chokeberry compared with the vitamin E treatment. Despite these large differences in one major antioxidant, only a numerical delay in oxidation was observed in the vitamin E group. Therefore, especially with chokeberry and sage, antioxidant substances (most likely the polyphenols) beyond the to-

copherols must have been provided to the hens to fully explain the increased oxidative stability of eggs and the corresponding slight increase in the meat. Some polyphenols are known for their antioxidant activity in vitro and their beneficial effect in preventing degenerative diseases in vivo [75]. Little is known about the exact metabolism, absorption, plasma half-life kinetics, uptake, and distribution among and within tissues for each of the many polyphenols [76]. Still, evidence exists that oxidative stress in vivo can be reduced [77]. Likewise, the plasma concentrations of certain polyphenols, namely quercetin, catechin, and naringenin, can be elevated by dietary intake [78]. Even less is known about the uptake and bioavailability of polyphenols by tissues. Ultimately, polyphenols are no longer considered to act as simple antioxidants in the body, but as important modulators of different cell signaling pathways (i.e., redox-sensitive transcription factors and the body's antioxidant defense system) [76]. As such, they could be more effective in adult animals than vitamin E, a hypothesis supported by the TBARS data in the present study.

The most astonishing finding of the present study was the great discrepancy between eggs and meat found in the antioxidant properties and tocopherol contents transmitted by the feed additives. As the herbal additives provided only about one-tenth of tocopherols compared with the vitamin E diet, a lower effect had been expected and the values could have been too low to result in clear increases in tocopherol levels in the meat but still be sufficient for enriching the eggs. However, even the vitamin E diet did not clearly increase the tocopherol level in the meat of the spent hens. Yet, it was effective in substantially enhancing egg tocopherol level. The plant additives also clearly increased egg tocopherol contents, especially when feeding sage. This indicates that antioxidants such as vitamin E are efficiently transferred to the body tissues of growing poultry such as broilers [16, 79], whereas in adult birds no large transfer of the same seems to occur any more. Still, the direct comparison by contrast analysis of the chokeberry and sage treatments against the control treatment revealed increased antioxidant protection also in the meat of spent hens. However, the small decrease in TBARS in the meat



**Figure 1.** Effect of vitamin E (40 mg/kg) and natural additives (2.5%) as feed supplements in comparison with an unsupplemented control treatment on the concentration of TBA reactive substances (mg of malondialdehyde/kg) values in lyophilized egg yolk powder and homogenized thigh meat from late-laying hens stored for different times (T = time point of measurement). For that purpose, yolk powder was stored at 20°C for 0, 4, 8, and 12 wk (T1–T4) and meat was stored at 4°C for 0, 3, 6, and 9 d (T1–T4). Values without common letters (a,b for dietary treatments; x,y for storage time) are significantly different ( $P < 0.05$ ) as calculated by the MIXED procedure of SAS [55], with storage time included as repeated measurement statement. Effects of storage time (meat T1–T4; each differs significantly), treatment, and the interaction of treatment  $\times$  storage time for egg yolk were significant ( $P < 0.001$ ). The interaction of treatment  $\times$  storage time for meat was not significant ( $P = 0.29$ ).

achieved by supplementation was far from being of practical relevance.

The discrepancy found in the transfer efficiency of antioxidants to egg and meat could be due to 3 reasons. First, the vitamin E depletion period of 2 wk could have been too short to allow differences in meat tocopherol content to be exhibited, explaining the lack of difference between the control and vitamin E treatment. Nevertheless, in the egg yolk, it was obvious that a possibility for substantial variation exists. This indicates that the supplementation level applied provided vitamin E beyond the body reserves of vitamin E; excluding the premise that simply no effect was seen because of the existing body vitamin E stores. Therefore, a second reason could have been that the compounds provided are not incorporated in existing body tissue as effectively as in newly generated body tissue. When the cells are formed, vitamin E is incorporated in the cellular lipid membrane as the main protector of the biological membrane from radical chain reactions and, thus, oxidation [80]. Furthermore, it is known that dividing cells have a lower level of lipid peroxidation, at least partly by incorporating more vitamin E [81]. With regard to the relevant muscles for meat production, intensive cell division and formation of new tissue occurs in growing poultry, whereas in adults, such as spent hens, cells only divide for homeostatic tissue turnover. Thus, the accumulation of vitamin E in the lipid membrane of muscular tissue is likely much lower in adults. However, the most relevant mechanism could have been that the known partitioning of nutrients toward the egg at cost of body tissues [2–4] in the laying hen indeed includes micronutrients and other bioactive compounds.

## CONCLUSIONS AND APPLICATIONS

1. A significant transfer of antioxidants from the supplements to the egg was observed, thus partially verifying hypothesis (1) tested in the experiment.
2. The efficiency to valorize spent hen meat by a specific late layer diet rich in antioxidants seems low. With the supplements tested an increase of the meat's oxidative stability, if any, was only achieved at a small magnitude. This finding disproves hypothesis (2).
3. Natural herbal additives with antioxidant properties may replace vitamin E in supporting the eggs in their antioxidant properties; this verified hypothesis (3). Sage and chokeberry exhibited clear antioxidant properties in egg yolk and were nearly as effective as the synthetic  $\alpha$ -tocopherol given at a level commonly supplemented.
4. Supplementing plants with antioxidants, especially those present in often discarded food industry by-products such as chokeberry pomace, would be a promising way to promote egg quality across the entire laying period. By contrast, their utility for increasing oxidative stability of the meat seems to be restricted to diets for growing poultry. Further research has to identify the most cost-effective ways to realize these applications.

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